and Arner, 2001). In this context it is fascinating that protein damage caused by H_2O_2-mediated oxidative stress is repaired by a system that is upregulated at the transcriptional level by this ROS.

However, the presence of epidermal MSRA is not sufficient to recover oxidized methionines, due to the production of both the (*R*) and the (*S*) diastereomers of methionine sulfoxide by H_2O_2-mediated oxidation. It therefore was not surprising to find highly expressed and functional MSRB in the epidermal compartment *in situ* as well as in keratinocytes, as shown in Figure 1 (K. Rübsam, ongoing medical thesis, University of Hamburg). Hence, it

> Skin aging is also associated with a decreased capacity to neutralize ROS

can be concluded that proteins and peptides damaged as a result of the oxidation of methionines in their sequences can be totally salvaged by MSRA and MSRB. In this context it is noteworthy that both TR and the MSRs express cytosolic and mitochondrial membrane-integrated isoforms, thus maintaining mitochondrial as well as cytosolic stability (Hansel *et al.*, 2002; Nordberg and Arner, 2001; Schallreuter and Wood, 2001).

The importance of epidermal TR/T for redox homeostasis

As early as in 1989 it was recognized that human epidermal keratinocytes express high levels of both cytosolic and membrane-integrated TR. This enzyme is subject to allosteric inhibition by calcium due to a single EF-hands binding site (for review see Schallreuter and Wood, 2001). Therefore, epidermal calcium homeostasis not only controls differentiation, but clearly also controls redox balance. The selenoenzyme TR, in addition to being the electron donor for both MSRs, this selenoenzyme has a very broad specificity, reducing disulfide bridges, H_2O_2, organoperoxides, vitamin K, and alloxan (Nordberg and Arner, 2001).

In summary, the discovery of the MSRs in the skin adds yet another important role to the epidermal antioxidant repair machinery. Moreover, the presence and function of these reductases imply a functional TR/T system. Whether the reductases themselves may also be targets for oxidative stress remains to be determined.

CONFLICT OF INTEREST

The author states no conflict of interest.

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"Out, Damned Spot!"

Richard A. Spritz[1]

Mice transgenic for the Kit Val620Ala mutation, which in humans has been associated with progressive piebaldism, exhibit dominant white spotting but show no evidence of progressive depigmentation. These results are consistent with the previous hypothesis that progressive piebaldism might result from digenic inheritance, of the *KIT*^{V620A} mutation that causes piebaldism and a second, unknown locus that causes progressive depigmentation.

Journal of Investigative Dermatology (2006) **126**, 949–951. doi:10.1038/sj.jid.5700220

Lady Macbeth's anguished lament speaks to some of the most puzzling conundrums in investigative dermatology. The white-spotting disorder piebaldism, because of its visually striking patches of white skin (leukoderma) and hair (poliosis), was known to the ancient Romans and is thought to be the first genetic disorder to have been recognized to exhibit autosomal dominant inheritance (Morgan, 1786). We have learned a lot over the centuries. We now know that the leukodermal patches result from an almost

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total lack of melanocytes from the involved regions. We now know that human piebaldism, and the homologous murine mutant "dominant white spotting" (*W*), result from loss-of-function and dominant-negative missense mutations in the *KIT* gene, and that there is a general correlation between genotype and severity of the resultant clinical phenotype (Spritz, 1994). We now know that *KIT* encodes the cell surface transmembrane tyrosine kinase receptor for KIT ligand (mast-cell growth factor; stem-cell factor; steel factor), and that KIT-dependent signaling plays key roles in melanogenesis, gametogenesis, and early stages of hematopoiesis. Nevertheless, the most obvious clinical questions remain unanswered. Why is developmental lack of melanocytes in piebaldism so localized, resulting in white spots rather than diffuse hypopigmentation? Why do the white spots preferentially involve the forehead, ventral chest and trunk, backs of the elbows, and fronts of the knees? Why are the congenital leukodermal patches of piebaldism so stable over a patient's life, neither filling in nor growing larger, whereas the acquired white spots of vitiligo may wax and wane? And why are the white spots of piebaldism utterly resistant to medical treatment, whereas those of vitiligo may fill in after ultraviolet therapy and immunotherapy?

> Why is developmental lack of melanocytes in piebaldism so localized?

Although the leukodermal patches of piebaldism typically lack melanocytes, they frequently contain hyperpigmented islands, abnormally pigmented macules, and freckles, all of which contain apparently normal melanocytes. Furthermore, the leukodermal patches often exhibit marginal hyperpigmentation, compared with the surrounding skin, which appears essentially normal. In human piebaldism, and in mice with "dominant white spotting" (*W*), the leukodermal patches are remarkably stable, usually being evident at birth and changing little over the course of patients' lives, although limited filling in has been reported in some milder cases of piebaldism (Davis and Verdol, 1976). Together, these phenomena suggest that deficient KIT-dependent signaling results in a primary defect of migration of neural crest melanoblasts to the skin during embryologic development and, furthermore, interferes with local migration of skin melanoblasts and melanocytes during postnatal life.

Recently, Richards and co-workers (2001) reported a mother and daughter with the remarkable phenotype of progressive piebaldism, the result of a novel amino acid substitution, Val620Ala (V620A), located in a highly conserved region of the KIT intracellular tyrosine kinase domain. In both mother and daughter, congenital skin and hair depigmentation progressed from infancy through at least mid-childhood, ultimately resulting in a relatively severe piebald phenotype. The biological basis for the highly atypical progressive clinical course in this family was not studied but would seem to imply reduced survival of skin melanocytes and self-renewing melanoblasts, in addition to defective melanoblast migration during development. Interestingly, several other family members had a tendency to localized hair graying but did not carry the *KIT* mutation. Accordingly, the authors speculated that progressive piebaldism in this family might result from digenic co-inheritance of the KIT^{V620A} mutation and an unknown dominant-acting unlinked mutation responsible for progressive hair graying.

In this issue, Tosaki *et al.* describe detailed investigation of the biology of the Kit^{V620A} substitution in transgenic mice, aiming to create an animal model for progressive piebaldism. Another *Kit* tyrosine kinase domain substitution, D790N, had been shown to act as a dominant-negative in transgenic mice (Ray *et al.*, 1991); this suggested that Kit^{V620A} might behave similarly, providing an animal model in which to study the biology of progressive melanocyte loss. The authors accordingly engineered the V620A substitution in mouse *Kit* complementary DNA, prepared four independent transgenic mouse lines, and carefully assessed the resultant phenotypes. Surprisingly, whereas all four transgenic lines exhibited dominant white spotting and reduction of mast-cell numbers consistent with the *W*-mutant phenotype, none of the lines showed any evidence of postnatal progression of the pigmentary phenotype. Thus, the Kit^{V620A} transgenic mice exhibit typical dominant white spotting but fail to model the most interesting aspect of the homologous human mutant phenotype, progressive depigmentation.

Tosaki and colleagues (2006) offer several hypotheses as to why the Kit^{V620A} transgenic mice do not manifest progressive depigmentation. They point out that KIT-dependent signaling is required both for developmental migration of melanoblasts to the skin and also for melanoblast/melanocyte maintenance and survival (Nishikawa *et al.*, 1991; Okura *et al.*, 1995; Kunisada *et al.*, 2001; Botchkareva *et al.*, 2001), and they imply that KIT^{V620A} might affect the latter more in humans than in mice. However, this would require that the KIT-V620A polypeptide exert a stable dysfunctional effect, in addition to or instead of acting as a straightforward dominant-negative. Perhaps more likely is the original hypothesis of Richards *et al.* (2001), that the family with progressive piebaldism segregated two unlinked mutations: KIT^{V620A}, causing typical dominant piebaldism, and a mutation in a second, unknown locus, causing progressive hair graying. Very recently, hair graying has indeed been linked to incomplete maintenance of self-renewing melanoblasts, in which Pax3 and Mitf seem to play key roles (Steingrimsson *et al.*, 2005). Although Richards and co-workers (2001) found no mutations of *MITF* in their original family, digenic inheritance involving *KIT* and some other locus remains an attractive hypothesis to account for progressive piebaldism in this family. Nevertheless, a full understanding of this phenomenon may await identification of genes specifically involved in hair graying in humans, and so, for now, atypical progressive piebaldism associated with KIT^{V620A} must be added to the list of conundrums, rather than to the list of puzzles solved.

CONFLICT OF INTEREST
The author states no conflict of interest.

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Lipid Rafts: Membrane Triage Centers

Xiao-qi Wang[1] and Amy S. Paller[1]

Both biochemical and live cell imaging studies suggest the existence of lipid rafts, specialized membrane microdomains that promote interaction among signaling molecules. Although their composition is still poorly understood, these highly dynamic domains are enriched in cholesterol, sphingolipids, and particular groups of proteins. The mechanism(s) by which trafficking into or out of lipid rafts affects signaling remains unclear.

Journal of Investigative Dermatology (2006) **126**, 951–953. doi:10.1038/sj.jid.5700282

Lipid rafts are compartmentalized membrane microdomains into which membrane lipids and proteins sort, allowing specific interactions that modulate signal transduction, membrane trafficking, cytoskeletal organization and motility, polarization, and pathogen entry (for reviews, see Rajendran and Simons, 2005; Pike, 2005). These membrane microdomains are thought to result from a tightly ordered lipid phase that is enriched in sterols (including cholesterol), as well as sphingolipids (including gangliosides) and phospholipids with saturated hydrocarbon chains. Although many cell surface proteins localize to the liquid disordered regions of the membrane, some proteins selectively partition into the lipid raft domains. These include glycosylphosphatidylinositol-anchored proteins (such as the urokinase-type plasminogen activator receptor), myristoylated or palmitoylated proteins (such as flotillin), doubly acetylated proteins (such as Src-family kinases), phospholipid-bound proteins (such as annexins), and cholesterol-bound transmembrane proteins (such as caveolins and hedgehog). Membrane microdomains are highly dynamic and possess considerable lateral mobility within the loosely ordered membrane.

Lipid rafts are defined by their resistance to extraction with nonionic detergents and their low density in sucrose gradients. Lipid rafts have been subdivided into caveolar and non-caveolar rafts that coexist in distinct membrane areas. Caveolar domains contain caveolae, stable flask-shaped invaginations of the plasma membrane that were recognized ultrastructurally more than 50 years ago. Caveolae are enriched in cholesterol and coated by the hairpin-like palmitoylated structural protein caveolin-1, which is thought to stabilize the invaginated caveolar structure. Non-caveolar lipid rafts are flat membrane microdomains that are also enriched in cholesterol and resistant to detergent extraction but have less caveolin-1 than caveolae. Unlike caveolar rafts, non-caveolar lipid rafts are heterogeneous in lipid and protein composition.

Problems associated with the study of lipid rafts

In initial studies, membrane components were extracted in 1% Triton X-100 at 4 °C and divided by centrifugation in a linear 5%–30% sucrose gradient. Milder detergents, such as Nonidet P-40, octylglucoside, CHAPS, Brij 58, and Brij 98, have more recently been used to isolate membranes, allowing the discovery of several proteins that are localized to lipid rafts but excluded by their Triton X-100 sensitivity. Rafts have also been isolated by detergent-free techniques (for example, use of a sodium carbonate buffer at alkaline pH or separation by serial gradients). The type of detergent, the temperature, the cell type, and the technique used for extraction influence phase behavior and protein composition. Thus, recovery of a group of lipids and proteins within the same fraction of detergent-resistant membrane does not, in itself, indicate colocalization within the cell membrane.

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